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Immunological Competence
and Chemical
Carcinogenesis

The Council for Tobacco Research - U.S.A., Inc.

110 East 59th Street
New York, N.Y. 10022
(212) 421-8885

FEB 3 1975

Contract
Application for Renewal of Research Grant

1. Principal Investigator:

(#1165)

Date: February 1, 1975

Richard A. Lerner, M.D., Member

2. Institution and Address:

Scripps Clinic and Research Foundation
476 Prospect Street
La Jolla, California 92037

3. Department where research will be done:

Department of Immunopathology

4. Short Title of Study:

Immunologic Competence and Chemical Carcinogenesis

5. Proposed renewal date:

July 1, 1975

6. How results to date have changed earlier specific research aims:

The specific research aim of this study continues to be the elucidation of the role played by the host immune mechanisms during chemical carcinogenesis.

In Phase I of the study we selected the best assay of immunocompetence. We then utilized this assay to establish the immunocompetence of both sexes of six strains of mice to five different antigens.

Phase II recently has been completed. It had previously been demonstrated that only strains which are so-called aryl hydrocarbon hydroxylase (AHH) inducible are susceptible to intratracheal (IT) chemical carcinogenesis by 3-methylcholanthrene (MCA). Essentially, we found that in these susceptible strains the IT administration of this carcinogen results in profound systemic immunosuppression. Phase III of the study will determine the importance of this immunosuppression in the development of lung tumors.

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7. How results to date have changed earlier working hypothesis:

There has been no change in earlier working hypothesis.

8. Any additional facilities now required?

No.

9. Any changes in personnel?

No.

10. Append outline of experimental protocol for ensuing year.

Our results from Phase II of this research demonstrated that in certain strains of mice 500 μ g MCA is immunosuppressive when given IT. This only occurs in strains of mice which are susceptible to induction of lung tumors by IT MCA.

The next series of experiments will attempt to dissect chemical carcinogenesis from immunosuppression in this system. This is important because the approach to the control of lung tumor induction would differ considerably depending upon whether the immunosuppression we observed was the permissive factor permitting tumor growth, or was merely a biologic event having no etiologic import.

Since C₃H strain mice are sensitive to induction of lung tumors with MCA administered IT and also exhibit significant immunosuppression, we will utilize mice of this strain exclusively. The first experiment will be directed towards finding a dose of carcinogen that is sufficient to induce lung tumors but does not cause immunosuppression.

Mice will be given intratracheally either saline, gelatin, 9.38 μ g, 18.75 μ g, 75 μ g or 300 μ g of MCA at Microbiological Associates, Inc. (MAI). Mice are coded as to dosage of carcinogen or control by marking specific toes, and the assays are performed at Scripps without knowledge of dosage of carcinogen administered (i.e., blind). They will then be shipped from MAI to Scripps Clinic and Research Foundation and six days after intratracheal inoculation, mice will be immunized with either goat erythrocytes or saline. Ten days later, the mice will be re-immunized, and either three, five, or seven days thereafter, the number of cells in each spleen secreting antibodies to goat erythrocytes will be assayed by utilizing the Jerne plaque assay. This will be performed on a random integrated schedule so that control and experimental animals are carefully co-mingled each day. The calendar for this study is attached. Each manipulation noted on the calendar involves fifty individual mice.

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This study will be completed by mid-year. The code will be broken and the data analyzed. We will then select a low dose of MCA, below the immunosuppressive threshold, and examine the immune response after multiple exposures to this low dosage.

Longer range plans are directed towards defining the role played by immunosuppression in permitting tumor development after chronic low dose exposure to chemical carcinogens and/or smoke.

11. List publications or papers in press resulting from this or closely related work.

Manuscript in preparation.

12. Summary progress report:

During the past year the second phase of this study was completed. This study was designed to determine whether or not doses of MCA sufficient to cause lung tumors when given IT also caused immunosuppression. Briefly, we found that in strains of mice that are sensitive to MCA carcinogenesis, systemic immunosuppression is observed after MCA is administered. Resistant strains of mice fail to exhibit systemic immunosuppression after MCA administration.

It has previously been shown that production of the aryl hydrocarbon hydroxylase (AHH) family of enzymes is induced by MCA, as well as other polycyclic aromatic hydrocarbons (PAH) in some strains of mice but not in others. The PAH are the substrate of these enzymes. MCA is only carcinogenic in the strains of mice in which it induces the AHH enzymes, and it is also only immunosuppressive in these strains of mice. Strains of mice that cannot catabolize MCA are not immunosuppressed by it, and do not develop tumors. This very important finding links immune competence to chemical carcinogenesis.

The following protocol was followed to arrive at the above conclusion. Goat erythrocytes (GRBC), with a mosaic of antigens on the surface, were selected as the test antigen. Three strains of mice were used (C₃H, DBA/2, C₅₇ Bl/6); two are AHH inducible; one is not. Three different histocompatibility types (H-2) are represented. Either MCA in gelatin, gelatin alone, or saline was administered IT. Six days later, mice were immunized with GRBC, and ten days after that were re-immunized. Individual assays to quantitate the number of cells making antibody to GRBC were performed 3, 5, 7 or 9 days after the secondary immunization. This kinetic approach eliminated the possibility that a delay in peak response induced by MCA would be mistaken for true suppression. For each animal immunized with GRBC, an identical animal was immunized with saline; this allowed us to detect so-called "natural" immunity. Since we studied the secondary response to an antigen (two immunizations) a normal response was conclusive evidence that both thymus dependent and bone marrow dependent lymphoid populations were functional in these mice.

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APPENDED CALENDARS

FOR WORK TO BE PERFORMED

AS DESCRIBED IN SECTION 10

1003536826

JANUARY 1975					1975				
MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY					
		1	2	3					
6	7	8	9	10					
13	14	15	16	17					
20	21	22	23	24					
27	28 Inoculation of MCA at Microbiological Associates, Inc. and ship to Scripps	29	30	31					

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FEBRUARY 1975				
MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
3 1° injection of mice treated 28 Jan	4	5 Inoculation of MCA at MAI and ship to Scripps	6	7
10	11 1° injection of 5 Feb mice ----- Inoculation of MCA at MAI and ship to Scripps	12	13 2° injection of 5 Feb mice	14
17 1° injection of 11 Feb mice	18 plaque assay of 28 Jan mice	19 Inoculation of MCA at MAI and ship to Scripps	20	21 2° injection of 5 Feb mice
24 plaque assay of 28 Jan mice	25 1° injection of 19 Feb mice ----- inoculation of MCA at MAI and ship to Scripps	26	27 2° injection of 11 Feb mice	28

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MARCH 1975				
MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
3 1° injection of 25 Feb mice	4 inoculation of MCA at MAI and ship to Scripps	5	6 plaque assay of 11 Feb mice	7 2° injection of 19 Feb mice
10 plaque assay of 19 Feb mice 1° injection of 4 March mice	11	12 inoculation of MCA at MAI and ship to Scripps	13 2° injection of 25 Feb mice	14
17	18 plaque assay of 25 Feb mice 1° injection of 12 March mice inoculation of MCA at MAI and ship to Scripps	19	20 2° injection of 4 March mice	21
24 1° injection of 18 March mice	25 inoculation of MCA at MAI and ship to Scripps	26	27 plaque assay of 4 March mice	28 2° injection of 12 March mice
31 plaque assay of 12 March mice 1° injection of 25 March mice		2		

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APRIL 1975				
MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
1° injection of 25 March mice		inoculation of MCA at MAI and ship to Scripps	2° injection of 18 March mice	
	plaque assay of 18 March mice 1° injection of 2 April mice inoculation of MCA at MAI and ship to Scripps		2° injection of 25 March mice	
1° injection of 8 April mice	inoculation of MCA at MAI and ship to Scripps		plaque assay of 25 March mice	2° injection of 2 April mice
plaque assay of 2 April mice 1° injection of 15 April mice		inoculation of MCA at MAI and ship to Scripps	2° injection of 8 April mice	
plaque assay of 12 March mice 1° injection of 25 March mice	plaque assay of 8 April mice 1° injection of 23 April mice inoculation of MCA at MAI and ship to Scripps			

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MAY 1975				
MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
			2° injection of 15 April mice	
1° injection of 29 April mice	inoculation of MCA at MAI and ship to Scripps		plaque assay of 15 April mice	2° injection of 23 April mice
plaque assay of 23 April mice 1° injection of 6 May mice			2° injection of 29 April mice	
plaque assay of 29 April mice	plaque assay of 29 April mice		2° injection of 6 May mice	
			plaque assay of 6 May mice	

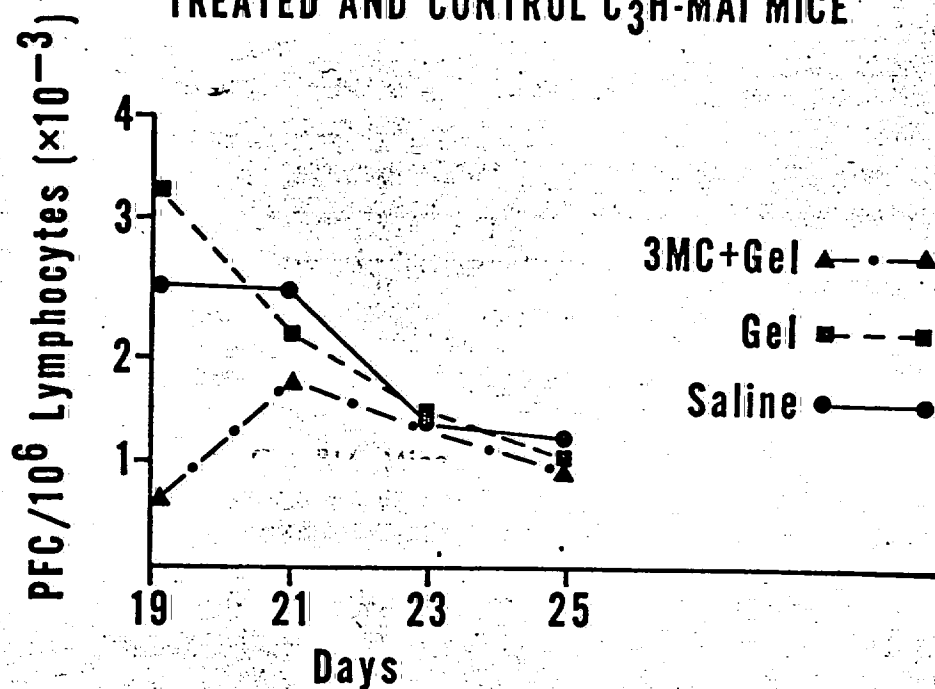
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GRAPHIC REPRESENTATION OF DATA
OBTAINED FROM STUDY OF IMMUNE COMPETENCE
IN MCA TREATED AND CONTROL MICE

-
- A. C_3H/F MAI Mice
B. $C_{57}Bl/6$ Mice
C. $DBA/2$ -J Mice
D. Composite of All Data

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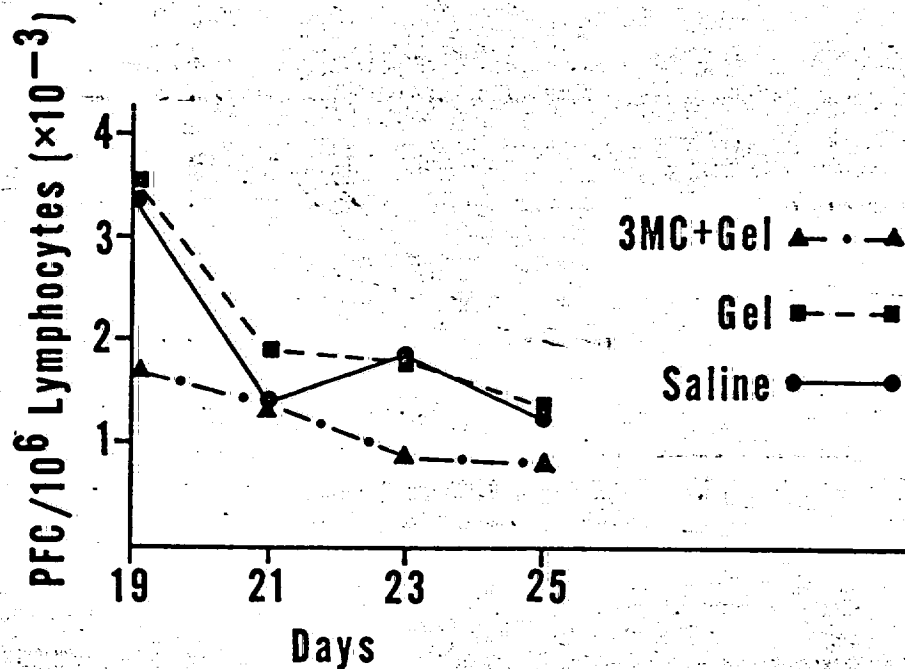
ANTI-GOAT ERYTHROCYTE PLAQUE FORMING CELLS
(INDIRECT) IN SPLEENS OF 3-METHYL CHOLANTHRENE
TREATED AND CONTROL C₃H-MAI MICE



GRAPHIC REPRESENTATION - A

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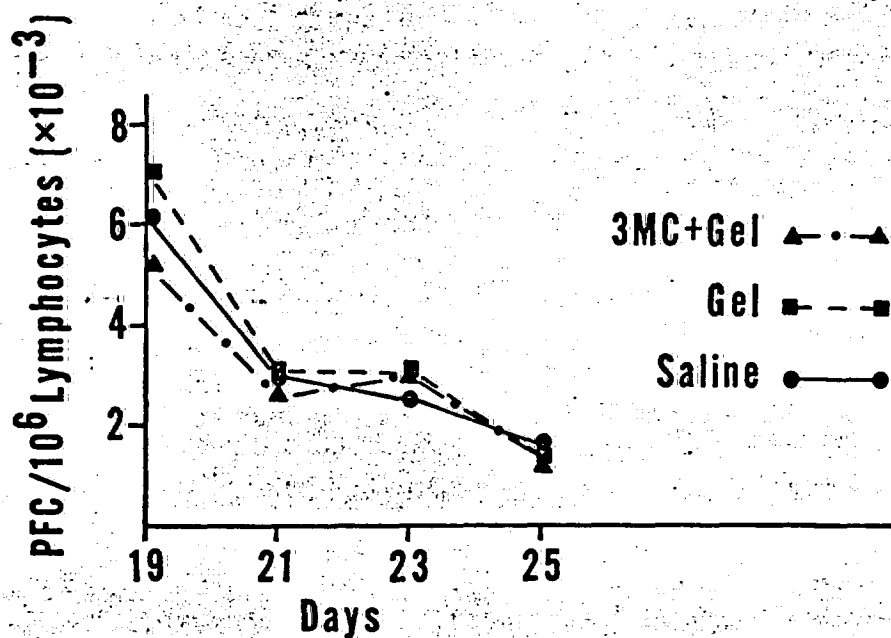
ANTI-GOAT ERYTHROCYTE PLAQUE FORMING CELLS
(INDIRECT) IN SPLEENS OF 3-METHYL CHOLANTHRENE
TREATED AND CONTROL C₅₇-BL/6-Cum MICE



GRAPHIC REPRESENTATION - B

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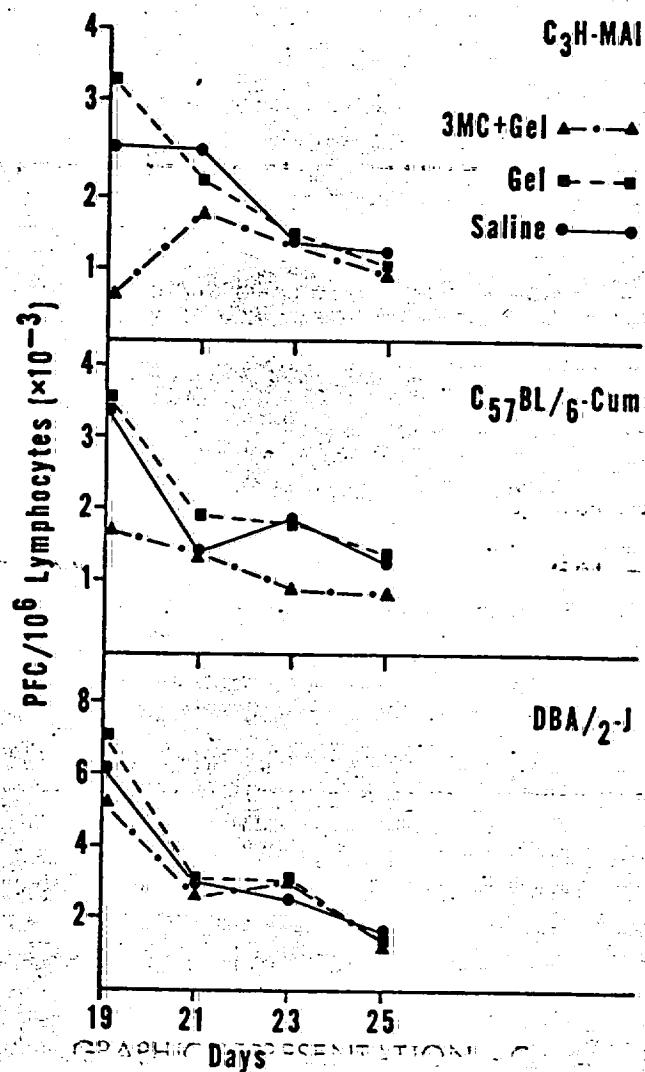
ANTI-GOAT ERYTHROCYTE PLAQUE FORMING CELLS
(INDIRECT) IN SPLEENS OF 3-METHYL CHOLANTHRENE
TREATED AND CONTROL DBA/2-J MICE



GRAPHIC REPRESENTATION - c

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ANTI-GOAT ERYTHROCYTE PLAQUE FORMING CELLS
(INDIRECT) IN SPLEENS OF 3-METHYL CHOLANTHRENE
TREATED AND CONTROL MICE



GRAPHIC REPRESENTATION - D

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TABULAR REPRESENTATION OF DATA
OBTAINED FROM STUDY OF IMMUNE COMPETENCE
IN MCA TREATED AND CONTROL MICE

Each number represents 10
individual assays. Background,
representing "natural" immunity,
has been subtracted for each
assay.

- A. Indirect (IgG) Plaque Forming Cells Per 10^6
spleen lymphocytes.
- B. Direct (IgM) Plaque Forming Cells Per 10^6
spleen lymphocytes.
-

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TABULAR REPRESENTATION - A

INDIRECT PFC/10⁶ SPLEEN LYMPHOCYTES

		<u>Day 13</u>	<u>Day 15</u>	<u>Day 17</u>	<u>Day 19</u>
	N	344	1227	1470	1089
C ₃ H	R	2828	2353	1553	1083
♀	L	2263	3157	1292	1284
	N	664	2218	899	552
C ₃ H	R	3692	2143	1391	864
♂	L	2946	1944	1363	1095
	N	6310	3018	3479	1441
DBA	R	6698	3681	3089	1685
♀	L	5157	3140	2727	1739
	N	3304	1679	2068	1302
DBA	R	7152	2127	3043	1035
♂	L	6898	2242	2094	1423
	N	1629	1404	1165	900
C ₅₇ Bl/6	R	3384	1890	2571	1242
♀	L	4364	1432	2186	1344
	N	1579	1238	555	640
C ₅₇ Bl/6	R	3558	1785	1061	1472
♂	L	2422	1285	1493	1089

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TABULAR REPRESENTATION - B

DIRECT PFC/ 10^6 SPLEEN LYMPHOCYTES

		<u>Day 13</u>	<u>Day 15</u>	<u>Day 17</u>	<u>Day 19</u>
C_3H ♀	N	198	82	215	81
	R	635	197	91	109
	L	519	172	243	171
C_3H ♂	N	366	124	77	59
	R	594	114	126	96
	L	563	216	79	102
DBA ♀	N	136	51	115	40
	R	109	76	104	77
	L	298	83	188	94
DBA ♂	N	93	41	27	43
	R	275	87	61	44
	L	423	447	58	170
$C_{57}^{Bl/6}$ ♀	N	638	139	78	80
	R	421	130	151	80
	L	734	101	127	71
$C_{57}^{Bl/6}$ ♂	N	511	115	56	109
	R	537	120	86	150
	L	575	186	124	110

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13. Budget for the coming year:

A. <u>Salaries:</u>		<u>% Time</u>	<u>Amount</u>
<u>Professional:</u>			
Lerner, Richard A.		15%	-0-
Levy, Richard L.		40%	9,280
<u>Technical:</u>			
Technicians (2)		100%	20,000
<u>Sub-Total for A</u>			<u>29,280</u>
B. <u>Consumable Supplies:</u>			
Supplies	11,000		
Animals	10,000		
<u>Sub-Total for B</u>			<u>21,000</u>
C. <u>Other Expenses:</u>			
Travel and Shipping Costs			
3,000			
Part time services of secretary, animal caretakers, photographer, histology technician, EM technician, electronic repairman and machinist			
4,000			
<u>Sub-Total for C</u>			<u>7,000</u>
<u>Running Total of A+B+C</u>			<u>57,280</u>
D. <u>Permanent Equipment:</u>			
None			
E. <u>Indirect Costs (15% of A+B+C)</u>			<u>8,592</u>
<u>TOTAL REQUEST</u>			<u>65,872</u>

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